

# FISH & RICHARDSON P.C.

A/REISSUE

225 Franklin Street  
Boston, Massachusetts  
02110-2804

Telephone  
617 542-5070

Facsimile  
617 542-8906

Web Site  
www.fr.com

November 4, 1998

Attorney Docket No.: 09125/001001

## Box Reissue

Assistant Commissioner for Patents  
Washington, DC 20231

Presented for filing is the application for reissue of U.S. Patent 5,583,114, issued on December 10, 1996.

Applicants: Thomas H. Barrows, Terry W. Lewis, Myhanh T. Truong and  
Matthew T. Scholz  
Title: ADHESIVE SEALANT COMPOSITION

Enclosed are the following papers, including those required to receive a filing date under 37 CFR §1.171 and 37 CFR §1.53(b):

Pages of Specification (including Abstract)	18
Claims	25
Declaration under 37 CFR §1.175	3
Drawing(s)	3
Consent of Assignee and Offer to Surrender	1
Request for Transfer of Original Drawings	1
Information Disclosure Statement	1
PTO 1449	3
Number of references cited	56

Check for \$100.00

"EXPRESS MAIL" Mailing Label Number EL182579374VS

Date of Deposit November 4, 1998  
I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office To Addressee" with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

Maureen T. Whalen  
Maureen T. Whalen

BOSTON

NEW YORK

SILICON VALLEY

SOUTHERN CALIFORNIA

TWIN CITIES

WASHINGTON, DC

11/04/98  
jc544  
D.S. PTO  
Frederick P. Fish  
1855-1930  
Richardson  
1859-1951

November 4, 1998

Page 2

Information Disclosure Statement	3
PTO 1449	2
Number of references cited (not enclosed)	23
Petition Under 37 CFR §1.324(a)	1
Statement Under 37 CFR §1.324(b)(1)	1
Statement Under 37 CFR §1.324(b)(2)	2
Statement Under 37 CFR §1.324(b)(3)	2
Transmittal of Certificate of Correction	
Request and Certificate of Correction	2
Declaration and Power of Attorney	2
Assignment Recordation Form and Assignment	2
Postcard	1
Basic filing fee	790.00
Total claims in excess of 20 times \$22.00	3,124.00
Independent claims in excess of 3 times \$82.00	246.00
Fee for multiple dependent claims	0.00
Total filing fee:	\$ 4,160.00

A check for the filing fee is enclosed. Please apply any other required fees or any credits to deposit account 06-1050, referencing the attorney docket number shown above.

If this application is found to be incomplete, or if a telephone conference would otherwise be helpful, please call the undersigned at 617/542-5070.

FISH & RICHARDSON P.C.

November 4, 1998

Page 3

Kindly acknowledge receipt of this application by returning the enclosed postcard.

Please send all correspondence to:

John J. Gagel  
Fish & Richardson P.C.  
225 Franklin Street  
Boston, MA 02110-2804

Respectfully submitted,

A handwritten signature in black ink, appearing to read "J. Gagel", with a large, stylized loop at the beginning.

John J. Gagel  
Reg. No. 33,499  
Enclosures  
331119.B11

## THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee : Minnesota Mining and Manufacturing Company  
Patent No.: 5,583,114  
Serial No.: 281,473  
Issued : December 10, 1996  
Filed : July 27, 1994  
Title : ADHESIVE SEALANT COMPOSITION

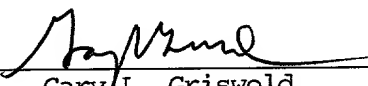
Assistant Commissioner for Patents  
Washington, DC 20231

CONSENT OF ASSIGNEE AND OFFER TO SURRENDER

The undersigned, Minnesota Mining and Manufacturing Company, being the assignee of all right, title and interest in and to the above-referenced U.S. Patent No. 5,583,114 by virtue of an assignment from Thomas H. Barrows, Terry W. Lewis and Myhanh T. Truong to Minnesota Mining and Manufacturing Company recorded on July 27, 1994, at Reel 7123, Frames 0460-0461, and an assignment from Matthew T. Scholtz to Minnesota Mining and Manufacturing Company, a copy of which is attached, hereby assents to the accompanying reissue application, and hereby offers to surrender said Patent 5,583,114 and further requests that Letters Patent be reissued to it upon the foregoing amended application.

Minnesota Mining and Manufacturing Company


Date: September 17,  
1998

By:   
Name: Gary L. Griswold  
Title: Staff Vice President & Chief Intellectual  
Property Counsel

303332.B11

"Express Mail" mailing label number EL1825793740S  
Date of Deposit November 4, 1998

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231

  
Maureen T. Whalen



US005583114A

## United States Patent [19]

Barrows et al.

[11] Patent Number: 5,583,114

[45] Date of Patent: Dec. 10, 1996

## [54] ADHESIVE SEALANT COMPOSITION

[75] Inventors: Thomas H. Barrows, Cottage Grove;  
Terry W. Lewis, Woodbury; Myhanh  
T. Truong, Blaine, all of Minn.

[73] Assignee: Minnesota Mining and  
Manufacturing Company, Saint Paul,  
Minn.

[21] Appl. No.: 281,473

[22] Filed: Jul. 27, 1994

[51] Int. Cl.<sup>6</sup> ..... A61K 38/00; A61K 39/00;  
C07K 1/00

[52] U.S. Cl. .... 514/21; 514/2; 514/4;  
530/362; 530/363; 530/366; 530/830; 424/77;  
424/78.02; 424/78.06; 424/179.1; 424/193.1;  
424/194.1; 424/422; 424/423; 424/428;  
525/54.1

[58] Field of Search ..... 514/21, 2, 4; 530/350,  
530/362, 363, 366, 830; 424/77, 78.02,  
78.06, 422, 423, 428; 524/54.1

## [56] References Cited

## U.S. PATENT DOCUMENTS

2,688,610	9/1954	Elins et al. ....	260/117
4,101,380	7/1978	Rubinstein et al. ....	530/350
4,163,097	7/1979	Baumann et al. ....	525/531
4,356,819	11/1982	Potaczek ..... 128/156	
4,416,814	11/1983	Battista ..... 260/117	
4,670,417	6/1987	Iwasaki et al. .... 514/6	
4,692,462	9/1987	Banerjee ..... 514/449	
4,839,345	6/1989	Doi et al. .... 514/21	
4,851,513	7/1989	Devore et al. .... 530/356	
5,162,430	11/1992	Rhee et al. .... 525/54.1	
5,385,606	1/1995	Kowanko ..... 106/124	

## FOREIGN PATENT DOCUMENTS

0194807A3	9/1988	European Pat. Off. .
2551660	3/1985	France .
WO90/13540	11/1990	WIPO .
WO92/02238	2/1992	WIPO .
WO94/03155	2/1994	WIPO .

## OTHER PUBLICATIONS

Abuchowshi et al., *The Journal of Biological Chemistry*, vol. 252, No. 11, pp. 3578-3581, Jun. 10, 1977.  
D'Urso et al., *Biotechnology Techniques*, vol. 8, No. 2, pp. 71-76, Feb. 1994.

Two-Component Fibrin Sealant Tisseel® Kit Brochure.

Abstract, Abdella et al., "A New Cleavable Reagent · Cross-linking and Reversible Immobilization of Protein *Biochem. Methods*, 91:16228v, 1979.

Kalman et al., "Synthesis of a Gene for Human Ser Albumin and Its Expression in *Saccharomyces cerevisiae* *Nucleic Acids Research*, vol. 18, No. 20, 1990, pp. 6075-6081.

Sleep et al., "The Secretion of Human Serum Albumin from the Yeast *Saccharomyces cerevisiae* Using Five Different Leader Sequences", *BioTechnology*, vol. 8, Jan. 1990, pp. 42-46.

Sijmons et al., "Production of Correctly Processed Human Serum Albumin in Transgenic Plants", *BioTechnology*, vol. 8, Mar. 1990, pp. 217-221.

Quirk et al., "Production of Recombinant Human Serum Albumin from *Saccharomyces cerevisiae*", *Biotechnology and Applied Biochemistry*, 11, 273-287 (1989).

D'Urso et al., "New Hydrogel Based on Polyethylene Glycol Cross-Linked with Bovine Serum Albumin", *Biotechnology Techniques*, vol. 8, No. 2 (Feb. 1994) pp. 71-76.

Primary Examiner—Elizabeth C. Weimar

Assistant Examiner—Abdel A. Mohamed

Attorney, Agent, or Firm—Gary L. Griswold; Walter N. Kirm; Paul W. Busse

[57]

## ABSTRACT

This invention is related to an adhesive composition which may be used to bond or seal tissue in vivo. The adhesive composition is readily formed from a two component mixture which includes a first part of a protein, preferably a serum albumin protein, in an aqueous buffer having a pH in the range of about 8.0-11.0 and a second part of a water-compatible or water-soluble bifunctional crosslinking agent. When the two parts of the mixture are combined, the mixture is initially a liquid which cures in vivo on the surface of tissue in less than about one minute to give a strong, flexible, pliant substantive composition which bonds to the tissue and is absorbed in about four to sixty days. The adhesive composition may be used either to bond tissue, to seal tissue or to prevent tissue adhesions caused by surgery.

17 Claims, 3 Drawing Sheets

## ADHESIVE SEALANT COMPOSITION

The present invention is generally related to an adhesive sealant composition which may be used to bond or seal tissue in vivo and is particularly related to a two component, liquid adhesive composition which is mixed together as it is applied to tissue and then cured in vivo in order to bond tissue, to seal tissue to prevent or control pulmonary system air leaks, or to prevent tissue adhesions caused by surgery.

## BACKGROUND

A variety of techniques have been used to bond or seal tissue. For example, different types of tissues have been mechanically bound or sealed with a number of procedures, materials and methods including sutures, staples, tapes and bandages. In some applications, these materials are made of absorbable materials which are intended to bond and/or seal tissue as it heals and then to be absorbed over a period of time.

The common use of a medical adhesive or "tissue glue" has not found widespread application. To date, some adhesive materials are known which may be used to adhere or stick tissue such as skin. For example, cyanoacrylate adhesives such as HISTOACRYL adhesive available from B. Braun, Melsungen, Germany or VETBOND tissue adhesive available from 3M, St. Paul, Minn. may be used to bond tissue. In addition to cyanoacrylate adhesives, other types of materials have been reported to adhere or stick to skin. For example, U.S. Pat. No. 4,839,345 to Doi et al. reports a hydrated crosslinked protein adhesive gel that is used as a cataplast or cosmetic mask that will externally adhere to skin but can be easily removed or pulled off and then readhered to the skin. Other crosslinked protein hydrogels have been reported to serve as a proteinaceous substrate to deliver therapeutic agents such as enzymes or drugs through skin or mucous membranes. See, for example, International Patent Application Ser. No. PCT/US93/07314 filed Aug. 4, 1993. Still other materials have been used as hemostatic agents to stop or prevent bleeding. In particular, mixtures of fibrinogen and thrombin such as TISSEEL sealant available from Immuno AG, Vienna, Austria or BERIPLAST-P hemostatic agent or sealant available from Behringwerke, Marburg, Germany, have been used in vascular surgery to seal tissue such as blood vessels and thus prevent blood leakage.

In sum, there are few available adhesive compositions that have sufficient strength, biocompatibility and bioabsorbability as well as other desired properties that would allow such compositions to be readily used in current medical procedures or practices. The unavailability of a suitable tissue adhesive or sealant may be related to the stringent requirements that a suitable, useful tissue adhesive must meet. Importantly, a tissue adhesive must provide substantial bonding strength for either internal or external tissues. The adhesive should be made of a biocompatible material which does not interfere with normal healing or regeneration processes. A suitable tissue adhesive must also be easily administered in a liquid form and then rapidly cured, ideally in less than a minute, once applied. In addition, a tissue adhesive must remain flexible, pliant and have good mechanical strength after being cured. Finally, a tissue adhesive must be completely absorbed or broken down in vivo, without producing an allergic response, adverse tissue reaction or systemic toxic effects, in an acceptable time period. Preferably a suitable adhesive would also be readily absorbed after it is applied.

## SUMMARY OF THE INVENTION

The present invention is a nontoxic, absorbable adhesive sealant composition which may be used to bond and/or seal tissue. The adhesive composition is readily formed from a two component mixture which includes a first part of a protein, preferably a serum protein such as albumin, in an aqueous buffer having a pH in the range of about 8.0-11.0 and a second part of a water-compatible or water-soluble bifunctional crosslinking agent. When the two parts of the mixture are combined, the mixture is initially liquid. The combined mixture then cures *in vivo* on the surface of tissue in less than about one minute to give a strong, flexible, pliant substantive composition which securely bonds to the tissue and is readily absorbed in about four to sixty days, preferably in about four to twenty-eight days.

In a preferred embodiment of the invention, an adhesive sealant composition is formed from a two part mixture that includes a proportion of a volume of a buffered basic serum albumin protein solution to a volume of a polyethylene glycol disuccinimidoyl succinate crosslinking agent in a range of from about 1:10 parts albumin solution by volume to about 10:1 parts by volume crosslinking agent. In order to facilitate the mixing of the two parts of the present adhesive composition, the volume to volume ratio of albumin solution to crosslinking agent is preferably a ratio of 1:1.

Preferred serum albumin proteins are selected to prevent adverse tissue or unwanted immunological responses. When the present adhesive mixture is used to bond or seal human tissue, a preferred serum albumin is purified human serum albumin which has been sterilized, dialyzed with a basic buffer having a pH value of about 8.0-11.0, concentrated by ultrafiltration through a membrane having about a 50,000 molecular weight cut-off to yield a concentrated, buffered aqueous mixture having about 20-60 wt/vol %, preferably about 35-45 wt/vol %, human serum albumin.

Preferred bifunctional crosslinking agents include polyethylene glycol derived crosslinking agents having a molecular weight (weight average) in a range of about 1,000-15,000 and preferably in a range of about 2,006-4,000. When the molecular weight of the crosslinking agent is in the range of about 1,000-5,000 the crosslinking agent is generally dissolved in water at a concentration of about 50-300 mg/ml. Similarly, when the molecular weight of the crosslinking agent is in the range of about 5,000-15,000 the crosslinking agent is generally dissolved in water at a concentration in the range of about 300-800 mg/ml.

The adhesive composition of this invention may be used in a variety of applications. Some applications include using the adhesive sealant composition to bind tissue together either as an adjunct to or as a replacement of sutures, staples, tapes and/or bandages. In another application, the present adhesive may be used to prevent post-surgical adhesions. In this application, the adhesive composition is applied and cured as a layer on surfaces of internal organs or tissues in order to prevent the formation of adhesions at a surgical site as the site heals. Additional applications include sealing tissues to prevent or control blood or other fluid leaks at suture or staple lines as well as to prevent or control air leaks in the pulmonary system.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graphical representation of a measured peel force of an adhesive composition of this invention.

FIG. 2 is a graphical representation of peel force measurements of different adhesive composition samples which are used to adhere excised guinea pig skin strips together.

FIG. 3 is a schematic diagram of an apparatus used to measure burst strength of an adhesive sealant composition.

#### DETAILED DESCRIPTION

The present invention is related to an adhesive composition which has high mechanical strength, flexibility, fast cure rate and sufficient adhesion needed to bond and/or seal tissue in vivo. The adhesive composition is made of two components, a buffered basic protein solution and a bifunctional crosslinking agent. The buffered protein solution and the bifunctional crosslinking agent are typically prepared using commercially available materials and established synthetic methods. The use of known, commercially available materials in the preparation of the adhesive composition provides a benefit in the practice of this invention because most of these materials generally have a history of clinical safety and/or use.

Suitable proteins for use in the present adhesive composition include nonimmunogenic, water soluble proteins. Serum lipoproteins are particularly well suited for this purpose because these proteins bind to lipids and also exhibit a relatively high elasticity in the natured or semi-natured state. These properties are believed to provide a cured matrix which is strong as well as pliant and elastic. Other soluble proteins, in addition to serum lipoproteins, are also suitable for use in the present invention. Aqueous mixtures of proteins such as derivatives of elastin, fibrinogen and collagen may be used in the present invention.

Preferred buffered protein solutions which may be used in the present adhesive composition include concentrated aqueous serum albumin protein mixtures that are buffered to a pH of between about 8.0–11.0 where the buffer concentration is in a range of about 0.01–0.25 molar. Suitable buffer systems include buffers which are physiologically and/or clinically acceptable such as known carbonate or phosphate buffer systems, provided the buffer does not adversely react with or otherwise alter the crosslinking agent. A preferred buffer system is a carbonate/bicarbonate buffer system at a pH value of about 9.0–10.5 at a concentration in the range of 0.05–0.15 molar.

Serum albumin protein is readily isolated from serum using known isolation processes. In addition, it is possible to produce human serum albumin from genetically transformed cells. See, for example, the reports of Quirk et al., *Biotechnology and Applied Biochemistry*, 11:273–287 (1989), Kalman et al., *Nucleic Acids Research*, 18:6075–6081 (1990), Sleep et al., *Biotechnology*, 8:42–46 (1990), and Sijmons et al., *Biotechnology*, 8:217–221 (1990). The ability to produce human serum albumin recombinantly provides the benefit that protein produced by this method will be free of pathogens, viruses or other contaminants that might contaminate albumin that is isolated directly from serum.

When used in the present buffered mixtures it has been found that the serum albumin is not denatured. Because the albumin is not denatured before it is used it is believed that the albumin proteins retain their natured, coiled conformation and thus, after being crosslinked during the curing process to provide a gel-like solid, the cured adhesive retains sufficient flexibility to provide a suitable adhesive matrix.

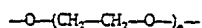
A variety of suitable crosslinking agents may be used in the present invention. Preferred crosslinking agents include a polyethylene glycol or polyoxyethylene chain portion (—PEG—), an activated leaving group portion (—G) and a linking moiety (—LM—) which binds the —PEG— portion



and the leaving group portion —G. Crosslinking agents include compounds of the formula



in which —PEG— is a diradical fragment represented by the formula



where a is an integer from 20–300; —LM— is also a diradical fragment such as a carbonate diradical represented by the formula, —C(O)—, a monoester diradical represented by the formula, —(CH<sub>2</sub>)<sub>b</sub>C(O)— where b is an integer from 1–5, a diester diradical represented by the formula, —C(O)—(CH<sub>2</sub>)<sub>c</sub>—C(O)— where c is an integer from 2–10 and where the aliphatic portion of the radical may be saturated or unsaturated, a dicarbonate represented by the formula —C(O)—O—(CH<sub>2</sub>)<sub>d</sub>—O—C(O)— where d is an integer from 2–10, or an oligomeric diradical represented by the formulas —R—C(O)—, —R—C(O)—(CH<sub>2</sub>)<sub>e</sub>—C(O)—, or —R—C(O)—O—(CH<sub>2</sub>)<sub>f</sub>—O—C(O)— where e is an integer from 2–10, d is an integer from 2–10, and R is a polymer or copolymer having 1–10 monomeric lactide, glycolide, trimethylene carbonate, caprolactone or p-dioxanone fragments; and —G is a leaving group such as a succinimidyl, maleimidyl, phthalimidyl, or alternatively, nitrophenyl, imidazolyl or tresyl leaving groups.

The —PEG— portion of the crosslinking agent is preferably derived from commercially available compounds having a weight average molecular weight in the range of about 1,000–15,000, preferably having a weight average molecular weight in the range of about 2,000–4,000. These compounds have been used in different types of biomedical materials because they have been demonstrated to be non-toxic as well as rapidly excreted from the body when the molecular weight is below about 30,000.

The leaving group, —G, portion of the crosslinking agent is an activated leaving group which allows the crosslinking agent to react or chemically bind to free primary or secondary amine groups of a protein. Suitable leaving groups include succinimidyl, other imides such as maleimidyl and phthalimidyl, heterocyclic leaving groups such as imidazolyl, aromatic leaving groups such as a nitrophenyl, or fluorinated alkylsulfone leaving groups such as tresyl (CF<sub>3</sub>—CH<sub>2</sub>—SO<sub>2</sub>—O—). A preferred leaving group is the succinimidyl group because studies of the mutagenicity, oncogenicity and teratogenicity of this group suggest that the small amount of this activating group which is released as the crosslinking reaction and/or the adhesive composition cures does not present a local or systemic toxicology risk.

When used in the present composition the linking moiety, —LM—, may be several different types of divalent compounds. For example, commercially available compounds having the —PEG— portion and the —G portion linked with a saturated dicarboxylic acid such as succinic acid to give a saturated diester linking moiety. Alternatively, an unsaturated dicarboxylic acid such as fumaric, maleic, phthalic or terephthalic acid may be used to give an unsaturated diester linking moiety. Alternatively, the linking moiety may be a readily hydrolyzable compounds such as oligomer derivatives of polylactic acid, polyglycolic acid, polydioxanone, polytrimethylene carbonate, or polycaprolactone as well as copolymers made using suitable monomers of these listed polymers.

In another embodiment of this invention an activated leaving group may be attached directly to a carbonate ester of polyethylene glycol. In this embodiment the linking

moiety, —LM—, would be a carbonate group, —C(O)— between the —PEG— and —G portions of the crosslinking agent. In still other embodiments of this invention the linking moiety may be a dicarbonate such as ethylene carbonate which is prepared by linking the —PEG and —G portions with ethylene bischloroformate.

The crosslinking agents may be prepared using known processes, procedures or synthetic methods such as the procedures reported in U.S. Pat. Nos. 4,101,380 or 4,839,345, the procedure reported in International Application Ser. No. PCT/US90/02133 filed Apr. 19, 1990 or the procedure reported by Abuchowski et al., *Cancer Biochem. Biophys.*, 7:175-186 (1984). Briefly, polyethylene glycol and a suitable acid anhydride are dissolved in a suitable polar organic solvent in the presence of base and refluxed for a period of time sufficient to form a polyethylene glycol diester diacid. The diester diacid is then reacted with a leaving group such as an N-hydroxy imide compound in a suitable polar organic solvent in the presence of dicyclohexylcarbodiimide or other condensing agents and stirred at room temperature to form the desired bifunctional crosslinking agent.

Alternatively, polyethylene glycol and a suitable dicarboxylic acid chloride or bischloroformate may be dissolved in a suitable polar organic solvent for a period of time sufficient to form the mixed acid chloride polyethylene glycol ester or mixed chloroformate polyethylene glycol ester. The mixed esters may then be reacted with a compound such as an N-hydroxy imide compound in a suitable polar organic solvent and stirred at an elevated temperature for a period of time sufficient to form the desired bifunctional crosslinking agent.

It has also been found that the cure time of the present adhesive compositions may be tailored by use of buffers having different pH values. For example, by varying the pH of the buffer it is possible to change the cure rate time from about 10 seconds to less than about 10 minutes. Briefly, mixing concentrated aqueous serum albumin and crosslinking agent mixtures with higher concentrations of buffer provides the fastest cure times. It has also been found that higher concentrations of protein and crosslinking agent provide a relatively stronger, cured matrix. However, if the mixtures are too concentrated and viscosity becomes too great, these adhesive compositions are not as readily applied or may provide adhesives with undesired properties. For example, mixtures which are too viscous may not be readily applied using available applicators such as syringes or spray apparatus. In addition, if the concentration of crosslinking agent is too high, the resulting cured adhesive matrix may swell to such an extent that the strength of the matrix in the presence of water or other fluids is lowered. Further, ability to adequately mix the two components using injecting and/or spraying apparatus may be reduced.

The two component adhesive composition of the present invention may be applied to tissue in a number of different ways. For example, the adhesive may be quickly mixed together and then applied using common applicators. Alternatively the two components may be mixed together and then applied as spray. In another application method, the two parts of the adhesive are added to a dual syringe. The two barrels of the syringe are attached to a "Y" connect which is fitted to a spiral mixer nozzle. As the two components are pressed out of the syringe, they are mixed in the nozzle and may be directly applied to the tissue as needed in a relatively uniform, controlled manner. Alternatively, a spray nozzle tip, such as a TISSEEL spray tip sold by Immuno AG, Vienna, Austria for use with a two-component fibrin sealant kit, may be used in place of the spiral mixer nozzle. In this

application, a fine spray of the adhesive composition is deposited on tissue as the plungers of the syringe are depressed.

The adhesive composition of the present invention may be used in a variety of current medical procedures and practices. In one application, the present adhesive composition may be used to eliminate or substantially reduce the number of sutures normally required using current practices as well as eliminate the need for subsequent removal of certain sutures. In another application, this adhesive composition may be used to attach skin grafts and to position tissue flaps or free flaps during reconstructive surgery. In still another application, this adhesive composition may be used to close gingival flaps in periodontal surgery. In all of these applications, the present adhesive composition is a thin layer of cured material which is effectively sandwiched between two adjacent layers of living tissues. Due to bioabsorbability and lack of toxicity of the adhesive composition, the healing and subsequent reattachment of the two layers of tissue to each other is not hampered.

In addition to the use of the present adhesive composition as an adhesive per se, the present composition may also be used as a sealant. When used in this application, this composition may be used to prevent air leaks now associated with pulmonary surgery or to inhibit or prevent bleeding in other surgical procedures. When used in this manner, the underlying tissue may be coated with a relatively thick layer of adhesive since the tissue itself needs to only heal on one side. The other side of the of the adhesive, when cured, simply presents a lubricous gel which will be absorbed in vivo in a relatively short period of time from about four to sixty days. In view of this property of the present adhesive composition, it may also be used to prevent unwanted tissue adhesions which are associated with current surgical procedures.

#### EXAMPLES

The following examples are intended to describe and illustrate the practice of the claimed invention. The examples, however, should not be construed to limit the scope of the present invention which is defined by the appended claims.

The following procedures were used to prepare several different types of bifunctional crosslinking agents. The following procedures are modifications of procedures reported in U.S. Pat. No. 4,101,380 and Abuchowski et al., cited above.

##### Example 1

##### Synthesis Of Polyethylene Glycol Disuccinimidyl Succinate PEG-SS2

Polyethylene glycol, PEG, (50 g, Aldrich Chemical Company, Milwaukee, Wis., sold as 3,400 average molecular weight, GPC analysis  $M_n$  was 2,980,  $M_w$  was 3,480) was dissolved in 1,2-dichloroethane (250 ml) containing succinic anhydride (14.7 g) and anhydrous pyridine (12 ml). The mixture was refluxed under nitrogen for three days. After filtration and evaporation of the solvent, the residue was dissolved in 100 ml water and treated with the cation exchange resin Dowex™ 50 ( $H^+$ ) (50 g) for 30 minutes. The mixture was then filtered and the Dowex™ 50 was washed with water (50 ml 1x). The combined filtrate was washed with anhydrous diethyl ether (50 ml 2x). The PEG-disuccinate was then extracted from the water phase with two 100

ml chloroform washes. Evaporation of chloroform yielded about 49 g of PEG-disuccinate.

The PEG-disuccinate was dissolved in 200 ml N,N-dimethylformamide (DMF) at 37° C. and 4.23 g of N-hydroxysuccinimide (NHS) were added to the solution. The mixture was cooled to 0° C. 7.58 g of dicyclohexylcarbodiimide (DCC) were dissolved in 50 ml DMF and added dropwise to the above solution with continuous stirring. The mixture was left at room temperature for 24 hours and filtered. 100 ml of toluene were added to the filtrate and the solution was placed in an ice bath. The desired polyethylene glycol disuccinimidyl succinate product, PEG-SS2, was precipitated by slowly adding petroleum ether. The precipitate was collected on a 10–20 micron sintered glass filter. Dissolution in toluene and precipitation with petroleum ether was repeated three times. The PEG-SS2 was further purified by dissolving in 100 ml of 0.1M pH 2.2 citrate/phosphate buffer and filtering through a 4–8 micron sintered glass filter. The PEG-SS2 was extracted with chloroform (100 ml 2x) and the solvent was evaporated under reduced pressure in a rotary evaporator. The PEG-SS2 was then dissolved in toluene and precipitated with petroleum ether, dried under vacuum overnight at room temperature, and stored in a refrigerator.

#### Example 2

##### Synthesis of N-hydroxysuccinimide Ester of Dicarboxymethyl Polyethylene Glycol

Dicarboxymethyl poly(ethylene glycol) (mol. wt. 3400) purchased from Shearwater Polymers, Inc., Huntsville, Ala. (5 g) and N-hydroxysuccinimide purchased from Sigma Chemical Co., St. Louis, Mo. (1 g) were dissolved in 30 ml of anhydrous DMF with mechanical stirring under nitrogen. The solution was cooled to 0° C. and a solution of dicyclohexylcarbodiimide (1.79 g) in 5 ml DMF was added dropwise. The stirring was continued in the cold for 3 hours then at room temperature overnight (16 hrs). Dicyclohexylurea which precipitated was removed by filtration. Toluene (100 ml) was added to the filtrate and cooled to 0° C. The product was then precipitated by addition of petroleum ether. The precipitate was collected on a sintered glass filter. Dissolution in toluene and reprecipitation with petroleum ether was repeated three times. The product was dried under vacuum in a desiccator.

#### Example 3

##### Synthesis of Polyethylene Glycol-di-oligoglycolide Disuccinimidyl Succinate

A 500 ml three neck round bottom flask was flame dried under nitrogen. 50 g of PEG (mol. wt. 3400), 300 ml of xylene, and 1 drop of 0.33M stannous octoate solution in xylene were charged into the flask with a continuous nitrogen purge. The flask was heated to boil the solution and 50 ml of xylene were removed by distillation. The solution was then cooled to room temperature. 17 g of glycolide (Boehringer Ingelheim KG, Ingelheim, Germany) was added to the flask and the reaction mixture was refluxed under nitrogen for 16 hours. The copolymer reaction mixture was filtered hot to remove polyglycolide homopolymer. The copolymer

then precipitated from the filtrate upon cooling and collected by filtration. The copolymer was placed in a flask with 500 ml of dichloromethane and 7 g of succinyl chloride. The solution was refluxed under nitrogen overnight (16 hours). 8.5 g of N-hydroxysuccinimide was added to the flask and refluxing was continued for another overnight period. A white solid was obtained by precipitation upon cooling the solution. The product was then purified by redissolving in toluene and reprecipitating with petroleum ether several times. The final precipitate was dried under vacuum and stored in a desiccator. The structure of the product was confirmed by NMR analysis.

#### Example 4

##### Synthesis of Polyethylene Glycol-dimaleimidyl Succinate

About 12 g of PEG-disuccinate and 1 g N-hydroxymaleimide (Aldrich Chemical Co.) were placed in a 250 ml three neck round bottom flask with 50 ml of anhydrous DMF under nitrogen. The mixture was dissolved at 60° C. with mechanical stirring and cooled to 0° C. A solution of 1.82 g dicyclohexylcarbodiimide in DMF (5 ml) was added dropwise to the flask. The reaction was allowed to mix overnight under nitrogen at room temperature. Dicyclohexylurea was removed by filtration and the product was obtained by adding toluene and precipitating with petroleum ether. Dissolution in toluene and reprecipitation with petroleum ether were repeated three times. The purified product was dried under vacuum and stored in a desiccator.

#### Example 5

##### Synthesis of Polyethylene Glycol-diphthalimidyl Succinate

About 15 g of PEG-disuccinate and 1.65 g N-hydroxyphthalimide (Aldrich Chemical Co.) were placed in a 250 ml three neck round bottom flask with 30 ml of anhydrous DMF under nitrogen. The mixture was dissolved at 60° C. with mechanical stirring and cooled to 0° C. A solution of 1.82 g dicyclohexylcarbodiimide in DMF (5 ml) was added dropwise to the flask. The reaction was allowed to mix overnight under nitrogen at room temperature. Dicyclohexylurea was removed by filtration and the product was obtained by adding toluene and precipitating with petroleum ether. Dissolution in toluene and reprecipitation with petroleum ether were repeated three times. The purified product was dried under vacuum and stored in a desiccator.

#### Example 6

##### Preparation of Two Component Adhesive

The following procedure was used to prepare a two-component adhesive using a variety of protein sources, and bifunctional crosslinking agents. Aqueous solutions of a protein and a crosslinking agent as listed in Table I were pipetted (0.2 ml of each solution) into a porcelain test well and mixed continuously with a stainless steel rod. The cure time and physical consistency of each of the two component adhesives are also listed in Table I.

The data indicated that fish and bovine gelatin, egg and serum albumin as well as casein protein crosslinked with PEG-SS2 provided an adhesive which was very elastic, had good adhesive strength and a relatively rapid cure rate.

TABLE 1

Protein	Bifunctional Crosslinking agent	Cure Time Consistency
Fish Gelatin Lot 23H0307 Sigma 40% 0.1 M pH 10 Carb/Bicarb	130 mg/ml PEG-SS2 3400 mw	40 sec Strong gel, very elastic, slightly sticky
Fish Gelatin Lot 23H0307 Sigma 40% 0.1 M pH 10 Carb/Bicarb	260 mg/ml PEG-SS2 3400 mw	40 sec Strong gel, very elastic, slightly sticky
Fish Gelatin Lot 23H0307 Sigma 40% 0.1 M pH 10 Carb/Bicarb	130 mg/ml PEG-SS2 10,000 mw	120 sec Soft gel, very sticky
Fish Gelatin Lot 23H0307 Sigma 40% 0.1 M pH 10 Carb/Bicarb	260 mg/ml PEG-SS2 10,000 mw	110 sec Soft gel to elastic, moderately sticky
Gelatin Bovine Skin Lot 53H0271 Sigma 40% 0.1 M pH 10 Carb/Bicarb	130 mg/ml PEG-SS2 3400 mw	40 sec Soft gel, not elastic
Gelatin Bovine Skin Lot 53H0271 Sigma 40% 0.1 M pH 10 Carb/Bicarb	260 mg/ml PEG-SS2 3400 mw	40 sec Soft gel, not elastic
Gelatin Bovine Skin Lot 53H0271 Sigma 40% 0.1 M pH 10 Carb/Bicarb	130 mg/ml PEG-SS2 10,000 mw	40 sec Soft gel, not elastic
Gelatin Bovine Skin Lot 53H0271 Sigma 40% 0.1 M pH 10 Carb/Bicarb	260 mg/ml PEG-SS2 10,000 mw	120 sec Soft gel, not elastic
Casein pH 9.4 12.6% Carb/Bicarb	130 mg/ml PEG-SS2 3400 mw	40 sec Strong gel, elastic, not sticky
Poly-L-Lysine 50 mg/ml H <sub>2</sub> O 300,000 mw Carb/Bicarb	130 mg/ml PEG-SS2 3400 mw	20 sec Waxy, no adhesive strength
Poly-L-Lysine 50 mg/ml H <sub>2</sub> O 300,000 mw Carb/Bicarb	260 mg/ml PEG-SS2 3400 mw	15 sec Waxy, no adhesive strength
Poly-L-Lysine 50 mg/ml H <sub>2</sub> O 300,000 mw Carb/Bicarb	130 mg/ml PEG-SS2 10,000 mw	10 sec Waxy, no adhesive strength
Poly-L-Lysine 50 mg/ml H <sub>2</sub> O 300,000 mw Carb/Bicarb	260 mg/ml PEG-SS2 10,000 mw	10 sec Waxy, no adhesive strength
Chicken Egg Albumin 40% 0.08 M pH 10 Carb/Bicarb	130 mg/ml PEG-SS2 3400 mw	210 sec soft, tacky
Rabbit Serum Albumin (RSA) Sigma Lot 19F9301 40% 0.1 M pH 10 Carb/Bicarb	130 mg/ml PEG-SS2 3400 mw	20 sec Very elastic, good adhesive strength, not sticky
Human Serum Albumin (HSA) Sigma Lot 63H9041 40% 0.1 M pH 10 Carb/Bicarb	130 mg/ml PEG-SS2 3400 mw	20 sec Very elastic, good adhesive strength, not sticky
HSA Sigma	130 mg/ml PEG-SS2 3400 mw	20 sec Very elastic, good adhesive

TABLE 1-continued

Protein	Bifunctional Crosslinking agent	Cure Time Consistency
Lot 63H9041 40% 0.1 M pH 8.44 Carb/Bicarb		strength, not sticky
HSA	260 mg/ml	10 sec Very elastic,
Sigma	PEG-SS2 3400 mw	good adhesive
Lot 63H9041 40% 0.1 M pH 8.44 Carb/Bicarb		strength, not sticky
HSA	130 mg/ml	30 sec Very elastic,
Sigma	PEG-SS2 10,000 mw	slight adhesive
Lot 63H9041 40% 0.1 M pH 8.44 Carb/Bicarb		strength, very sticky
HSA	260 mg/ml	25 sec Very elastic,
Sigma	PEG-SS2 10,000 mw	slight adhesive
Lot 63H9041 40% 0.1 M pH 8.44 Carb/Bicarb		strength, very sticky
HSA	130 mg/ml	20 sec Turned brown
Baxter Healthcare	PEG-dimaleimidyl	upon curing,
Corp.	succinate	hard gel, not
Lot 2837A238AA Carb/Bicarb	Example 4	sticky
HSA	130 mg/ml	10 sec Turned red upon
Baxter	PEG-dipthalimidyl	curing, hard gel,
Lot 2837A238AA	succinate	not sticky
Carb/Bicarb	Example 5	
HSA	130 mg/ml	8 sec Hard gel, not
Baxter	PEG-dicarbonylmethyl	sticky, no color
Lot 2837A238AA	disuccinimidyl	change
Carb/Bicarb	Example 2	
HSA	130 mg/ml	40 sec Hard gel, not
Baxter	PEG-diethylglycolide	sticky, no color
Lot 2837A238AA	disuccinimidyl succinate	change
Carb/Bicarb	Example 3	
HSA	130 mg/ml	30 sec Hard gel, not
Baxter	PEG-disuccinimidyl	sticky, no color
Lot 2837A238AA	propionate	change
Carb/Bicarb	PEG(SPA)2	
HSA	260 mg/ml	40 sec Hard gel, not
Baxter	PEG-disuccinimidyl	sticky, no color
Lot 2837A238AA	propionate	change
Carb/Bicarb	PEG(SPA)2	
HSA	130 mg/ml	48 hrs Hard gel, not
Baxter	PEG-dioxycarbonyl	(cure) sticky, no color
Lot 2837A238AA	imidazole	change
Carb/Bicarb	PEG(CDI)2	
HSA	130 mg/ml	140 sec Hard gel, not
Baxter	PEG-dinitrophenyl	sticky, changed
Lot 2837A238AA	carbonate	to bright yellow
Carb/Bicarb	PEG(NPC)2	color
HSA	260 mg/ml	140 sec Hard gel, not
Baxter	PEG-dinitrophenyl	sticky, changed
Lot 2837A238AA	carbonate	to bright yellow
Carb/Bicarb	PEG(NPC)2	color
HSA	130 mg/ml	8 hrs Hard gel, not
Baxter	PEG-diethylate	(viscous) sticky, no color
Lot 2837A238AA	PEG(tres)2	24 hrs change
Carb/Bicarb		(cure)
HSA	130 mg/ml	72 hrs Hard gel, not
Baxter	PEG-diglycidyl ether	(cure) sticky, no color
Lot 2837A238AA	PEG(epox)2	change
Carb/Bicarb		
HSA	130 mg/ml	no cure Liquid
Baxter	PEG-dialdehyde	
Lot 2837A238AA	PEG(alq)2	
Carb/Bicarb		

mw = weight average molecular weight

## Example 7

## Effect of Buffer and pH

Two component adhesives were prepared according to the process described in Example 6 except that the pH of the buffer in the protein solution was changed as listed in Table 2. The data indicate that a preferred pH range is about 8.44-10.0.

TABLE 2

Protein	Crosslinking agent PEG-SS2	Cure Time	Consistency
HSA Baxter Lot 2837A238AA 40% 0.1 M pH 7.4 Carb/Bicarb	130 mg/ml 3400 mw	10 min	Initially softer adhesive, hardens with aging
HSA Sigma Lot 63H9041 40% 0.1 M pH 8.44 Carb/Bicarb	130 mg/ml 3400 mw	20 sec	Very elastic, good adhesive strength, not sticky
HSA Sigma Lot 63H9041 40% 0.15 M pH 9.07 Carb/Bicarb	130 mg/ml 3400 mw	10 sec	Hard gel, not sticky
HSA Sigma Lot 63H9041 40% 0.2 M pH 9.52 Carb/Bicarb	130 mg/ml 3400 mw	5 sec	Hard gel, not sticky
HSA Sigma Lot 63H9041 40% 0.2 M pH 9.52 Carb/Bicarb	260 mg/ml 3400 mw	5 sec	Hard gel, not sticky
HSA Sigma Lot 63H9041 40% 0.2 M pH 9.52 Carb/Bicarb	130 mg/ml 10,000 mw	7 sec	Elastic to hard gel, slightly sticky
HSA Sigma Lot 63H9041 40% 0.2 M pH 9.52 Carb/Bicarb	260 mg/ml 10,000 mw	7 sec	Elastic to hard gel, slightly sticky
HSA Baxter Lot 2837A238AA 40% 0.1 M pH 10 Carb/Bicarb	130 mg/ml 3400 mw	25 sec	Very elastic, not sticky
HSA Sigma Lot 63H9041 40% 0.1 M pH 10 Carb/Bicarb	130 mg/ml 3400 mw	25 sec	Very elastic, not sticky

mw = weight average molecular weight

## Example 8

## Effect of Crosslinking Agent on Adhesive Strength

A 30% HSA (Human Serum Albumin) solution from Sigma Chemical Co. and a 25% HSA solution from Baxter Healthcare, Inc. were dialyzed against 0.1M carbonate/bicarbonate pH 10 buffer at 4° C. overnight and concentrated to about 40% by ultra-filtration through a 50,000 molecular weight cut-off cellulose ester disc membrane (Spectrum Medical Industries, Inc.) in a pressure filtration cell under nitrogen at 60 psig. The final concentration was calculated based on the volume of collected filtrate. The maximum concentration obtained under these conditions during overnight ultra-filtration was typically 42-45%. The RSA (Rabbit Serum Albumin) from Sigma and RSA crystallized



protein from ICN Biomedical, Inc. were dissolved in 0.1M pH 10 carbonate/bicarbonate buffer and concentrated to 40% by the same method used for HSA.

Various concentrations of PEG-SS2 (3,400 mw and 10,000 mw) were prepared in deionized water. The albumins and crosslinking agent solutions were delivered in equal volume using a 1 ml dual syringe. The syringe tips were fitted with a Y connector which connected to a specially machined TEFLON adaptor inserted into a 1.8 in.x0.187 in. (4.57 cmx0.475 cm) dia. spiral mixer nozzle (TAH Industries, Inc., Robbinsville, N.J., part no. 150-312). The adhesive mixture was injected through the mixer directly onto the test substrate for adhesion testing.

Freshly excised guinea pig skin was cut into strips and a polystyrene window with an opening of 0.5x1.0 inches (1.27 cmx2.54 cm) was placed on one end of the strip to contain the glue in a specific region. Upon filling the window with glue it was covered with another strip of guinea pig skin. A 500 g steel weight was placed on top of this assembly for about one minute. The sample was peeled apart in the jaws of a computer controlled mechanical testing machine (880 Material Test System, MTS System, Inc., Minneapolis, Minn.) set at a strain rate of 0.8 in./min. (2 cm/min.) with a gage length of 1 in. (2.54 cm) and a 5 lbs. (2.27 kg) load cell. Peel force was recorded after the initiation of adhesive failure as the constant force require to continue peeling as shown in FIG. 1. Four replicates were performed for each test condition. The results of this test are listed in FIG. 2.

#### Example 9

##### Measurement of Adhesive Sealant Burst Strength

A pressurization assembly illustrated in FIG. 3 was used to test the bursting strength of materials used to seal standardized holes or slits in test membranes. This assembly included an aluminum pressure vessel (1) having a 35 mm inside diameter fitted with a millivolt output type pressure transducer (2) with a range of 0 to 15 psig (MODEL PX236, Omega Engineering, Inc., Stamford, Conn.) and a pressure inlet port (3). To perform a test, about a 5 mm diameter hole (4) (or other standardized defect) was cut in the center of a test membrane (5) using a die cutter. The membrane was then placed on a piece of 0.4 mm thick TEFLON film with the hole in the membrane centered in a larger (14 mm diameter) hole in the TEFLON film. The TEFLON film was then placed on a flat surface with the membrane side down and adhesive sealant test material was applied to fill the hole in the film. A solid TEFLON block was then quickly placed over the sealant prior to cure so that the TEFLON film served as a spacer to create a layer of sealant exactly 0.4 mm thick. After the desired cure time elapsed, the TEFLON block was inverted and the membrane was carefully peeled off to obtain a circular patch of sealant (6) covering the hole in the membrane. The test membrane with sealed defect was then mounted onto the open end of the pressure vessel (7) by placing it between two rubber washers (8) and then between two metal washers (9). An air tight seal was obtained by screwing the threaded cover (10) onto the matching threads (11) of the pressure vessel. The opening in the cover (12) was also 35 mm in diameter which, in combination with the 35 mm inside diameter washers, provided a fixed membrane surface area for pressure testing.

Two types of membranes were used, either a collagen membrane or a freshly excised porcine pericardium sample. The porcine pericardium sample was either used immediately upon harvest or after storage in a moisture-proof container at 4° C. for no longer than 24 hours. Under these

conditions there was no discernible difference in sealant performance based on storage time of that tissue.

The pressurization sequence was initiated by injecting air into the pressure inlet at a fixed rate of one cubic centimeter per second using a syringe pump (Sage Instruments Model 351, Orion Research, Inc.). The pressure transducer was connected to a digital strain gauge meter (Omega Model DP205-S, Omega Engineering, Inc.) programmed to read pressure (mm mercury) and to display the peak pressure value at the time of adhesive sealant rupture. Replicate tests gave reproducible peak pressure values and the standard deviation was reported in each case.

Pressure tests were performed with an adhesive composition of 40% HSA (or RSA) in 0.08M carbonate/bicarbonate buffer at different pH values with 3,400 m.wt. PEG-SS2 (130 mg/ml) on collagen and pericardium membranes. The results listed in Table 3 demonstrate excellent sealant performance with typical peak pressure values of about 130 mm Hg.

In addition, the peak pressure for the above sealants after soaking in saline solution was measured. The test was performed as described above except that the surface of the sealant coated membrane was flooded with saline for up to a time period of 90 minutes before pressurization. Although the sealant hydrogel swelled to about double in thickness, substantial retention of sealant performance was retained.

Table 4 shows the data obtained by testing a variety of proteins including fish skin gelatin, chicken egg albumin, and fibrinogen. Fibrinogen mixed with thrombin ("fibrin glue", BERIPLAST-P sealant, Behringwerke, Marburg, Germany) was also used as a control sealant material. None of these materials performed as well as the serum albumin examples. The main disadvantage was the cure and aging time required to achieve significant strength. In particular, chicken egg albumin required twenty-five minutes of post cure aging to achieve the same burst strength obtained from serum albumin aged for less than five minutes.

The same process was repeated for additional 25% HSA solutions by dialyzing against 0.08M carbonate/bicarbonate buffers at pH 9 and pH 8. A pH 7 solution of HSA was obtained by concentration of the original 25% HSA solution to 40% by ultrafiltration. The crosslinking agent solution PEG-SS2 (3400 mw) was 130 mg dissolved in one ml deionized water. The albumin and crosslinking agent solutions were delivered in equal volume using a one ml dual syringe as in Example 8. The pressure tests were performed as above using collagen membrane except that the sealant hydrogel was aged before testing. The results are also listed in Table 4. These data demonstrate that optimal pressure test values are achieved faster with increasing pH of the albumin solution. Moreover, the resultant cured sealant obtained after complete curing has taken place is unexpectedly higher with higher pH of the albumin solution.

TABLE 3

Tissue	Tissue Opening	Adhesive Composition	Burst Pressure (mm Hg)
Collagen	4.56 mm dia. hole	HSA:PEG-SS2	150
Collagen	5 mm slit	HSA:PEG-SS2	112
Collagen	4.56 mm dia. hole	RSA:PEG-SS2	130
Collagen	5 mm slit	RSA:PEG-SS2	125
Portine	4.56 mm dia. hole	HSA:PEG-SS2	155
Pericardium			
Portine	5 mm slit	HSA:PEG-SS2	130
Pericardium			
Porcine	4.56 mm dia. hole	RSA:PEG-SS2	125
Pericardium			
Porcine	5 mm slit	RSA:PEG-SS2	130
Pericardium			

TABLE 4

Pressure Test of Different Proteins Using Collagen and Pericardium  
HSA: 40% 0.08 M Carb/Bicarb Buffer in Saline Lot #2837a328AA  
RSA: 40% 0.08 M Carb/Bicarb Buffer in Saline Lot #62-451-0050 INC  
PEG-SS2: 3400 mw lot #103128-110 (130 mg/ml)  
Defect: 4.56 mm hole  
Air Flow Rate: 1 cc/s

Protein	Crosslinker	Membrane	Pressure (mm Hg)		Comments
			Ave	Stdev	
HSA pH 10	PEG-SS2	Collagen	149	9	No bubbles
		Pericardium	154	4	5 min after curing
		Pericardium	196	5	10 min after curing
HSA pH 10	PEG-SS2	Collagen	144		5 min after curing
			155		10 min after curing
			162		20 min after curing
HSA pH 9	PEG-SS2	Collagen	108		5 min after curing
			114		10 min after curing
			116		20 min after curing
HSA pH 8	PEG-SS2	Collagen	36		5 min after curing
			78		10 min after curing
			90		20 min after curing
HSA pH 7	PEG-SS2	Collagen	30		10 min after curing
			52		20 min after curing
RSA pH 10	PEG-SS2	Collagen	134	5	No bubbles
		Pericardium	126	10	5 min after curing
		Pericardium	194	9	10 min after curing
Fish Gelatin pH 10 40% (Sigma)	PEG-SS2	Collagen	34	2	10 min after curing

TABLE 4-continued

Pressure Test of Different Proteins Using Collagen and Pericardium					
HSA: 40% 0.08 M Carb/Bicarb Buffer in Saline Lot #2837a328AA					
RSA: 40% 0.08 M Carb/Bicarb Buffer in Saline Lot #82-451-0050 INC					
PEG-SS2: 3400 mw lot #103128-110 (130 mg/ml)					
Defect: 4.56 mm hole					
Air Flow Rate: 1 cc/s					
Protein	Crosslinker	Membrane	Pressure (mm Hg)		Comments
			Ave	Stdev	
Chicken Egg Albumin pH 10 40% (Sigma) Fibrin Glue (BERIPLAST-P) Used according to mfg. instructions	PEG-SS2	Collagen	14	3	10 min after curing
			151	5	45 min after curing
		Pericardium	8	2	5 min after curing with saline, glue slid off easily
Bovine Fibrinogen pH 10 15% (Sigma)	PEG-SS2	Collagen	39	2	5 min after curing without saline, leaked underneath
			8	2	5 min after curing
			8	2	60 min after curing, glue slid off easily

## Example 10

## Use of a Two Component Adhesive Sealant in General and Thoracic Surgery

An anesthetized pig was used as an experimental model for thoracic surgical complications such as staple line leaks during lung and bronchus resections, bronchopleural fistulas, and other conditions resulting in pneumothorax.

The two component adhesive included Part A, a 40% HSA prepared by dialysis of commercially available HSA (25% Solution, BUMINATE 25%, Baxter Healthcare Corp., Hyland Division, Glendale, Calif.) against 0.08M pH 10 carbonate/bicarbonate buffer followed by concentration to 40% by ultrafiltration at 50 psi using a 50,000 molecular weight cut-off cellulose ester disc membrane and Part B, a 130 mg/ml solution of 3,400 m.wt. PEG-SS2 dissolved in sterile distilled water no more than 30 minutes prior to use. The PEG-SS2 was synthesized and purified as described in Example 1.

A stab wound was made on the lung of an anesthetized pig with a scalpel which resulted in significant air leakage during inspiration as evidenced by bubbling of air through irrigation fluid administered to the site. The wound was blotted with gauze to remove blood and fluid. The respirator was turned off and the adhesive was applied as a sealant using a dual syringe (Behring PANTAJECT syringe, Behringwerke, Marburg, Germany) equipped with a spiral mixing tip. After a 20 second cure time ventilation was restored and the lung was again covered with irrigation fluid. No air leaks were observed.

A functional end-to-end anastomosis in pig intestine was conducted using a standard stapling procedure. The adhesive material described above was applied to the staple lines. This resulted in a clear, adherent hydrogel coating which appeared to seal the anastomotic line.

Under these conditions it was observed that anastomotic lines coated with the sealant were air tight whereas anastomotic lines not sealed were not air tight.

#### Example 11

##### Use of Two Component Adhesive to Prevent Post-Surgical Adhesions

The tissue sealant hydrogel tested was a two part liquid system. Part A was a sterile 40% (w/v) solution of human serum albumin in isotonic pH 10 carbonate buffer (0.1M). Part B was a 400 mg/ml solution of 10,000 molecular weight PEG-SS2 (polyethylene glycol disuccinimidyl succinate) in sterile distilled water prepared just prior to use. Solutions A and B were mixed in equal volumes with a dual syringe system connected to a static mixing head (Tah Industries, Inc.).

Post-surgical adhesion prevention evaluation of this sealant formulation was initiated in a series of ten female rabbits. A 2x2 cm area of the abdominal wall was excised down to the fascia on each side of the abdominal cavity exposed by a midline laparotomy incision. The uterine horns were injured by scraping 20 times with a no. 10 scalpel blade. Each animal served as its own control by randomly applying test material to only one of the abdominal wall injuries. The uterine horns were then attached with two stitches to the abdominal wall within a few millimeters of the edge of the wound closest to the laparotomy incision.

Two weeks after surgery the rabbits were examined in order to evaluate and score the extent, type, and tenacity of adhesions present on the abdominal wall injury sites. These results are shown in Table 5. The rating system used to obtain these scores is shown in Table 6. Although technical difficulties were encountered as noted in Table 5, the test material clearly provided an unexpected benefit in both the prevention of adhesions and a reduction in their severity without the presence of a known active ingredient.

TABLE 5

Scoring of Adhesions Formed in Material Evaluation						
Animal	Characteristic					
	Extent		Type		Tenacity	
	Control	Treatment	Control	Treatment	Control	Treatment
BAM 8	2	0+	3	0+	3	0+
BAM 9	3	1	3	1	3	1
BAM 10	0+	1	0+	3	0+	2
BAM 11	0*	0	0*	0	0*	0
BAM 12	4	4	3	3	3	3
BAM 13	2	1	3	2	3	2
BAM 14	1*	0	3*	0	3*	0
BAM 15	1	0**	1	0**	2	0**
BAM 16	1	0*	1	0*	2	0*
BAM 17	1	0*	1	0*	2	0*
Average	1.5	0.7	1.5	0.9	2.1	0.8

\*Uterine horn tacked to abdominal wall with only one suture

\*\*Uterine horn no longer sutured to abdominal wall

+ Fascia removed with peritoneum and muscle layers

TABLE 6

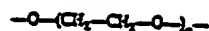
Adhesion Scoring	
Characteristic	Adhesion Score
Extent (% sidewall involvement)	
None	0
≤25	1
≤50	2
≤75	3
>75	4
Type	
None	0
Filmy, no vessels (transparent)	1
Opaque, no vessels (translucent)	2
Opaque, small vessels present grossly	3
Opaque, larger vessels present grossly	4
Tenacity	
None	0
Adhesions essentially fell apart	1
Adhesions lysed with traction	2
Adhesions required sharp dissection for lysis	3

We claim:

1. An adhesive composition consisting essentially of
  - i) a first aqueous mixture of about 20-60 wt/vol % serum albumin in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0,
  - ii) a second aqueous mixture of about 50-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 1,000-15,000, wherein the crosslinking agent is of the formula



wherein —PEG— is a diradical fragment represented by the formula



where a is an integer from 20-300;

wherein —LM— is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, —C(O)—, a monoester diradical of the formula, —(CH<sub>2</sub>)<sub>b</sub>C(O)— where b is an integer from 1-5, a

diester diradical of the formula, —C(O)—(CH<sub>2</sub>)<sub>c</sub>—C(O)— where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, a dicarbonate diradical of the formula —C(O)—O—(CH<sub>2</sub>)<sub>d</sub>—O—C(O)— where d is an integer from 2-10, and an oligomeric diradical represented by the formulas —R—C(O)—, —R—C(O)—(CH<sub>2</sub>)<sub>e</sub>—C(O)—, or —R—C(O)—O—(CH<sub>2</sub>)<sub>f</sub>—O—C(O)— where e is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone and p-dioxanone; and

wherein —G is a leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl or tressyl, and

wherein a combination of the first and second mixtures is initially liquid and then cures on the surface of tissue to give a flexible, substantive matrix which bonds to the tissue and has a burst strength greater than about 10 mmHg.

2. The adhesive mixture of claim 1 wherein the protein in the first mixture is about 35-45 wt/vol % serum albumin.

3. The adhesive composition of claim 1 wherein the serum albumin is human serum albumin.

4. The adhesive composition of claim 1 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

5. The adhesive composition of claim 1 wherein the second aqueous mixture is about 50-300 mg/ml of a crosslinking agent having a molecular weight in a range of about 1,000-5,000.

6. The adhesive composition of claim 1 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

7. The adhesive composition of claim 1 wherein —LM— is an oligomeric diradical —R—C(O)—(CH<sub>2</sub>)<sub>e</sub>—C(O)— where e is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone and p-dioxanone.

8. The adhesive composition of claim 1 wherein —G is succinimidyl.

9. An in vivo method of adhering tissue comprising the steps of topically applying and bonding an adhesive mixture of claim 1 to the tissue.

10. An in vivo method of sealing air leaks in pulmonary tissues comprising the step of topically applying and curing the adhesive mixture of claims 1 to an air leak site in the pulmonary tissue.

11. An in vivo method to prevent post-surgical adhesions comprising the step of topically applying and curing the adhesive mixture of claims 1 to tissue surrounding a surgical site.

12. An in vivo method to seal tissue comprising the step of topically applying and bonding the adhesive mixture of claims 1 to tissue to prevent or control blood or other fluid leaks.

13. The adhesive composition of claim 1 wherein the second aqueous mixture is about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

14. The adhesive composition of claim 13 wherein —LM— is a diester diradical of the formula —C(O)—(CH<sub>2</sub>)<sub>2</sub>—C(O)—.

15. The adhesive mixture of claim 1 wherein —LM— is a diester diradical of the formula, —C(O)—(CH<sub>2</sub>)<sub>c</sub>—C(O)— where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

16. The adhesive composition of claim 15 wherein —LM— is an oligomeric diradical derived from polyglycolic acid.

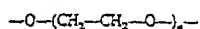
17. A method of making a tissue adhesive consisting of the step of forming a mixture of

- i) a first aqueous mixture of about 20-60 wt/vol % serum albumin in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0,
- ii) a second aqueous mixture of about 50-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 1,000-15,000, wherein the crosslinking agent is of the formula





wherein  $-PEG-$  is a diradical fragment represented by the formula



where  $a$  is an integer from 20-300;

wherein  $-LM-$  is a diradical fragment selected from the group consisting of a carbonate diradical of the formula,  $-C(O)-$ , a monoester diradical of the formula,  $-(CH_2)_bC(O)-$  where  $b$  is an integer from 1-5, a diester diradical of the formula,  $-C(O)-(CH_2)_c-C(O)-$  where  $c$  is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, a dicarbonate diradical of the formula  $-C(O)-O-(CH_2)_d-O-C(O)-$  where  $d$  is an integer from 2-10, and an oligomeric diradical represented by the formulas  $-R-C(O)-$ ,  $-R-C(O)-(CH_2)_e-C(O)-$ , or  $-R-C(O)-O-(CH_2)_d-O-C(O)-$  where  $c$  is an integer from 2-10,  $d$  is an integer from 2-10, and  $R$  is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone and p-dioxanone; and

wherein  $-G$  is a leaving group selected from the group consisting of succinimidyl, malcimidyl, phthalimidyl, imidazolyl, nitrophenyl or tresyl, and

wherein a combination of the first and second mixtures is initially liquid and then cures on the surface of tissue to give a flexible, substantive matrix which bonds to the tissue and has a burst strength greater than about 10 mmHg.

\* \* \* \* \*

18. A method of treating tissue to prevent or control air or fluid leaks comprising:

providing a composition to tissue, said composition including an albumin protein and a crosslinking agent, said crosslinking agent having a polyoxyethylene chain portion  
5 and an activated leaving group which allows the crosslinking agent to react with said protein; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured  
10 matrix.

19. The method of claim 18 wherein said composition is cured to produce the matrix in less than about 10 minutes.

20. The method of claim 18 wherein said composition is cured to produce the matrix in less than about one  
15 minute.

21. The method of claim 18 wherein said composition is cured to produce the matrix in about ten seconds.

22. The method of claim 18 comprising providing the composition to the tissue using a syringe.  
20

23. The method of claim 18 comprising providing the composition to the tissue using a dual syringe.

24. The method of claim 18 comprising providing the composition to the tissue using a spray apparatus.

25. The method of claim 18 wherein the matrix is resorbed.  
25

26. The method of claim 25 wherein the matrix is resorbed in about four to sixty days.

27. The method of claim 18 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

28. The method of claim 18 comprising curing said composition to provide a cured matrix that has a burst pressure greater than about 10 mmHg.

29. The method of claim 27 wherein the matrix has a burst pressure of about 34 mmHg or greater.

30. The method of claim 29 wherein the matrix has a burst pressure of about 90 mmHg or greater.

31. The method of claim 30 wherein the matrix has a burst pressure of about 130 mmHg or greater.

32. The method of claim 18 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-15,000.

33. The method of claim 32 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

34. The method of claim 18 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

35. The method of claim 34 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

36. The method of claim 18 further comprising  
 5 mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,  
wherein the first mixture includes about 20-60  
wt/vol% of the protein in about 0.01-0.25 molar buffer at a  
pH in a range of about 8.0-11.0 and the second mixture  
 10 includes about 50-800 mg/ml of the crosslinking agent having  
a molecular weight in a range of about 1,000-15,000.

37. The method of claim 36 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

15 wherein:

-PEG- is a diradical fragment represented by the formula

-O-(CH<sub>2</sub>-CH<sub>2</sub>-O-)<sub>a</sub>-

where a is an integer from 20-300;

20 -LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, -(CH<sub>2</sub>)<sub>b</sub>C(O)- where b is an integer from 1-5, a diester radical of the formula, -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where  
 25 the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula -C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O-C(O)- where d is an integer from 2-10, or an oligomeric diradical represented by the formulas  
-R-C(O)-, -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)-, or -R-C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O- where  
 30 c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments

selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

38. The method of claim 37 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

39. The method of claim 38 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

40. The method of claim 37 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

41. The method of claim 37 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

42. The method of claim 37 wherein -LM- is an oligomeric diradical  $-R-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

43. The method of claim 37 wherein -G is succinimidyl.

44. The method of claim 37 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

5 45. The method of claim 37 wherein -LM- is a diester diradical of the formula  $-C(O)-(CH_2)_2-C(O)-$ .

46. The method of claim 37 wherein -LM- is a diester diradical of the formula  $-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

10 47. The method of claim 37 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

48. The method of claim 18 comprising treating tissue to prevent or control a fluid leak.

15 49. The method of claim 48 wherein the fluid leak is a blood leak.

50. The method of claim 18 wherein the tissue includes an air leak.

51. The method of claim 50 wherein the air leak is in the pulmonary system.

20 52. A method of treating tissue to prevent formation of an adhesion comprising:  
providing a composition to tissue, said composition including an albumin protein and a crosslinking agent, said crosslinking agent having a polyoxyethylene chain portion

and an activated leaving group which allows the crosslinking agent to react with said protein; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix.

53. The method of claim 52 wherein said composition is cured to produce the matrix in less than about 10 minutes.

54. The method of claim 52 wherein said composition is cured to produce the matrix in less than about one minute.

55. The method of claim 52 wherein said composition is cured to produce the matrix in about ten seconds.

56. The method of claim 52 comprising providing the composition to the tissue using a syringe.

57. The method of claim 52 comprising providing the composition to the tissue using a dual syringe.

58. The method of claim 52 comprising providing the composition to the tissue using a spray apparatus.

59. The method of claim 52 wherein the matrix is resorbed.

60. The method of claim 59 wherein the matrix is resorbed in about four to sixty days.

61. The method of claim 52 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

5 62. The method of claim 52 comprising curing said composition to provide a cured matrix that has a burst pressure greater than about 10 mmHg.

63. The method of claim 62 wherein the matrix has a burst pressure of about 34 mmHg or greater.

10 64. The method of claim 63 wherein the matrix has a burst pressure of about 90 mmHg or greater.

65. The method of claim 64 wherein the matrix has a burst pressure of about 130 mmHg or greater.

15 66. The method of claim 52 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-15,000.

67. The method of claim 66 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

20 68. The method of claim 52 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

69. The method of claim 68 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.



70. The method of claim 52 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue, wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

71. The method of claim 70 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

wherein:

-PEG- is a diradical fragment represented by the formula

-O-(CH<sub>2</sub>-CH<sub>2</sub>-O)-<sub>a</sub>-

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, -(CH<sub>2</sub>)<sub>b</sub>C(O)- where b is an integer from 1-5, a diester radical of the formula, -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula -C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O-C(O)- where d is an integer from 2-10, or an oligomeric diradical represented by the formulas -R-C(O)-, -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)-, or -R-C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O- where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

5 72. The method of claim 71 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

73. The method of claim 72 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

10 74. The method of claim 71 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

75. The method of claim 71 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

15 76. The method of claim 71 wherein -LM- is an oligomeric diradical  $-R-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone,  
20 and p-dioxanone.

77. The method of claim 71 wherein -G is succinimidyl.

25 78. The method of claim 71 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

79. The method of claim 71 wherein -LM- is a diester diradical of the formula  $-C(O)-(CH_2)_2-C(O)-$ .

80. The method of claim 71 wherein -LM- is a diester diradical of the formula  $-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

81. The method of claim 71 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

82. The method of claim 52 wherein the composition is provided to tissue at a surgical site.

83. The method of claim 52 wherein the composition is provided on a surface of an internal organ.

84. A method of treating tissue to bind layers of tissue together comprising:

providing a composition to tissue, said composition including an albumin protein and a crosslinking agent, said crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix.

85. The method of claim 84 wherein said composition is cured to produce the matrix in less than about 10 minutes.

86. The method of claim 84 wherein said composition is cured to produce the matrix in less than about one minute.

5 87. The method of claim 84 wherein said composition is cured to produce the matrix in about ten seconds.

88. The method of claim 84 comprising providing the composition to the tissue using a syringe.

89. The method of claim 84 comprising providing the composition to the tissue using a dual syringe.

10 90. The method of claim 84 comprising providing the composition to the tissue using a spray apparatus.

91. The method of claim 84 wherein the matrix is resorbed.

15 92. The method of claim 91 wherein the matrix is resorbed in about four to sixty days.

93. The method of claim 84 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

20 94. The method of claim 84 comprising curing said composition to provide a cured matrix that has a burst pressure greater than about 10 mmHg.

95. The method of claim 94 wherein the matrix has a burst pressure of about 34 mmHg or greater.

96. The method of claim 95 wherein the matrix has a burst pressure of about 90 mmHg or greater.

97. The method of claim 96 wherein the matrix has a burst pressure of about 130 mmHg or greater.

5        98. The method of claim 84 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-15,000.

10       99. The method of claim 98 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

100. The method of claim 84 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

15       101. The method of claim 100 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

102. The method of claim 84 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

20       wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

25       103. The method of claim 102 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

wherein:

-PEG- is a diradical fragment represented by the formula

5 -O-(CH<sub>2</sub>-CH<sub>2</sub>-O)-<sub>a</sub>-

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, -(CH<sub>2</sub>)<sub>b</sub>C(O)- where b is an integer from 1-5, a diester radical of the formula, -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula -C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O-C(O)- where d is an integer from 2-10, or  
 15 an oligomeric diradical represented by the formulas -R-C(O)-, -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)-, or -R-C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O- where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and  
 20 -G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

25 104. The method of claim 103 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

105. The method of claim 104 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

106. The method of claim 103 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

5 107. The method of claim 103 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

10 108. The method of claim 103 wherein -LM- is an oligomeric diradical  $-R-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

109. The method of claim 103 wherein -G is succinimidyl.

15 110. The method of claim 103 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

111. The method of claim 103 wherein -LM- is a diester diradical of the formula  $-C(O)-(CH_2)_2-C(O)-$ .

20 112. The method of claim 103 wherein -LM- is a diester diradical of the formula  $-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

25 113. The method of claim 103 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

114. The method of claim 84 wherein the matrix binds tissue together in addition to a suture, a staple, a tape, or a bandage.

5 115. The method of claim 84 wherein the composition is provided to attach skin grafts.

116. The method of claim 84 wherein the composition is provided to attach adjacent layers of tissue.

117. The method of claim 84 wherein the composition is provided to position tissue flaps.

10 118. The method of claim 84 wherein the composition is provided to close gingival flaps.

119. A method of treating tissue comprising:  
providing a composition to tissue, said composition including an albumin protein and a crosslinking agent, said  
15 crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein; and  
curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured  
20 matrix.

120. The method of claim 119 wherein said composition is cured to produce the matrix in less than about 10 minutes.

25 121. The method of claim 119 wherein said composition is cured to produce the matrix in less than about one minute.



122. The method of claim 119 wherein said composition is cured to produce the matrix in about ten seconds.

5 123. The method of claim 119 comprising providing the composition to the tissue using a syringe.

124. The method of claim 119 comprising providing the composition to the tissue using a dual syringe.

125. The method of claim 119 comprising providing the composition to the tissue using a spray apparatus.

10 126. The method of claim 119 wherein the matrix is resorbed.

127. The method of claim 126 wherein the matrix is resorbed in about four to sixty days.

15 128. The method of claim 119 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

129. The method of claim 119 comprising curing said composition to provide a cured matrix that has a burst pressure greater than about 10 mmHg.

20 130. The method of claim 94 wherein the matrix has a burst pressure of about 34 mmHg or greater.

131. The method of claim 95 wherein the matrix has a burst pressure of about 90 mmHg or greater.

132. The method of claim 96 wherein the matrix has a burst pressure of about 130 mmHg or greater.

5 133. The method of claim 119 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-15,000.

134. The method of claim 133 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

10 135. The method of claim 119 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

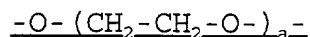
136. The method of claim 135 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

15 137. The method of claim 119 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,  
wherein the first mixture includes about 20-60  
20 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

138. The method of claim 137 wherein the crosslinking agent is of the formula

25 G-LM-PEG-LM-G  
wherein:

-PEG- is a diradical fragment represented by the formula



where a is an integer from 20-300;

- 5 -LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, -(CH<sub>2</sub>)<sub>b</sub>C(O)- where b is an integer from 1-5, a diester radical of the formula, -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where  
 10 the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula -C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O-C(O)- where d is an integer from 2-10, or an oligomeric diradical represented by the formulas -R-C(O)-, -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)-, or -R-C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O- where  
 15 c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and  
 20 -G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

139. The method of claim 138 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

25 140. The method of claim 139 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

141. The method of claim 138 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

142. The method of claim 138 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

5 143. The method of claim 138 wherein -LM- is an oligomeric diradical  $-R-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

10 144. The method of claim 138 wherein -G is succinimidyl.

145. The method of claim 138 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

15 146. The method of claim 138 wherein -LM- is a diester diradical of the formula  $-C(O)-(CH_2)_2-C(O)-$ .

20 147. The method of claim 138 wherein -LM- is a diester diradical of the formula  $-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

148. The method of claim 138 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

149. The method of claim 119 comprising curing the composition on the tissue to seal the tissue.

150. The method of claim 149 comprising treating tissue to prevent or control a fluid leak.

151. The method of claim 150 wherein the fluid leak is a blood leak.

5        152. The method of claim 149 wherein the tissue includes an air leak.

153. The method of claim 152 wherein the air leak is in the pulmonary system.

10       154. The method of claim 119 wherein the composition is provided to tissue at a surgical site.

155. The method of claims 119 comprising curing the composition at the tissue to prevent a tissue adhesion.

156. The method of claim 119 wherein the composition is provided on a surface of an internal organ.

15       157. The method of claim 119 comprising curing the composition to form a matrix to bind tissue.

158. The method of claim 157 wherein the matrix binds tissue together in addition to a suture, a staple, a tape, or a bandage.

20       159. The method of claim 119 wherein the composition is provided to attach skin grafts.

160. The method of claim 119 wherein the composition is provided to attach adjacent layers of tissue.

161. The method of claim 119 wherein the composition is provided to position tissue flaps.

162. The method of claim 119 wherein the composition is provided to close gingival flaps.

[illegible]

• •

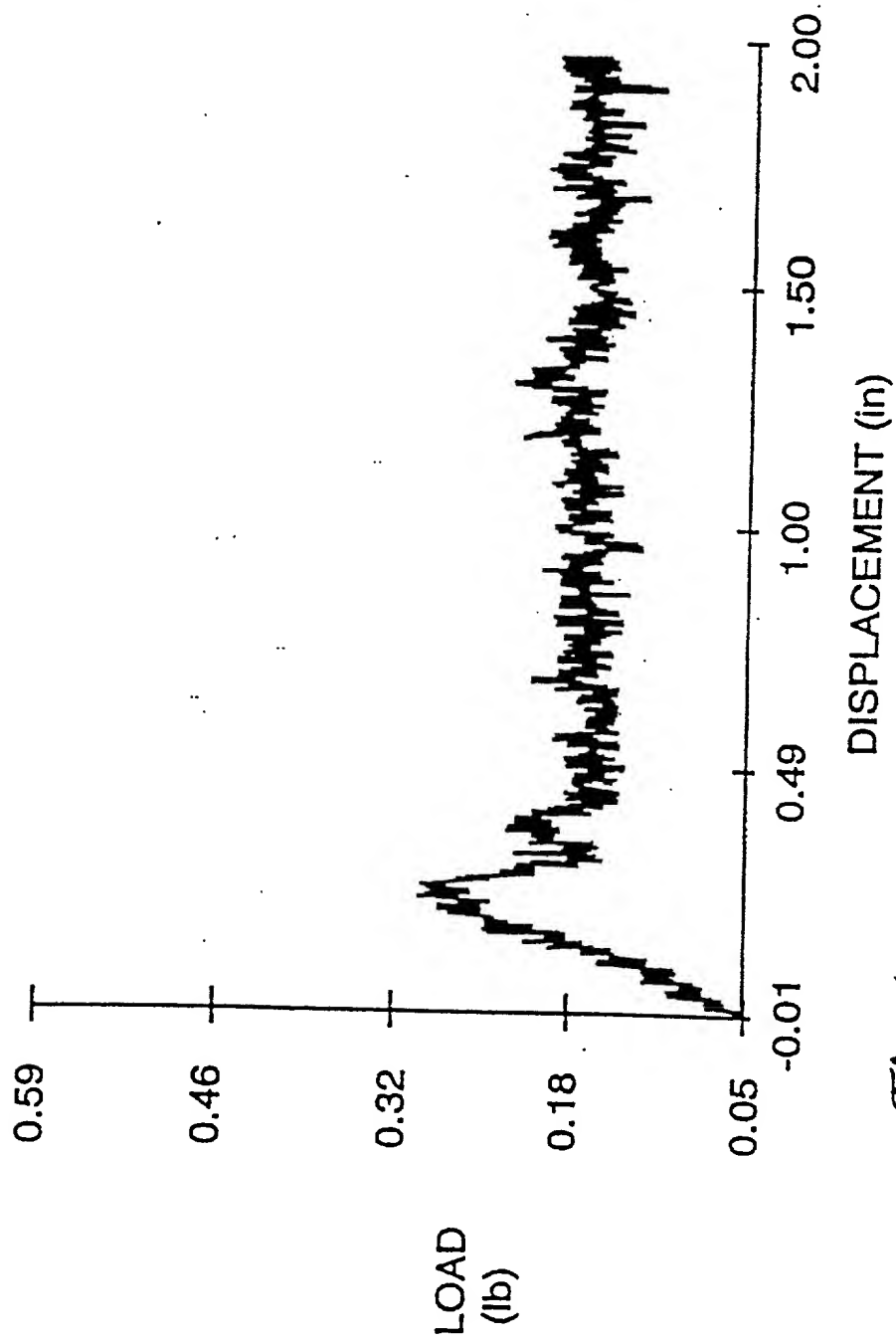
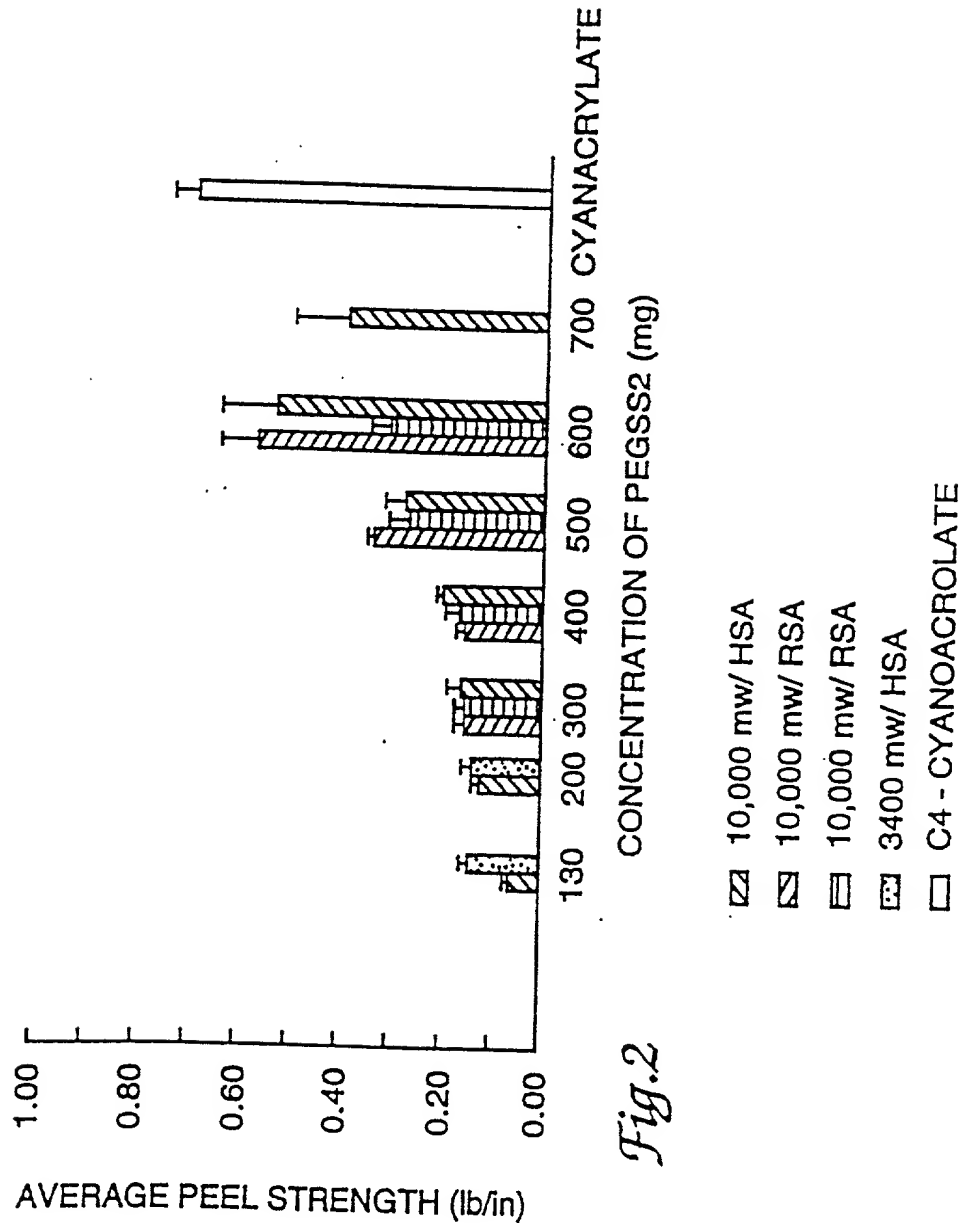
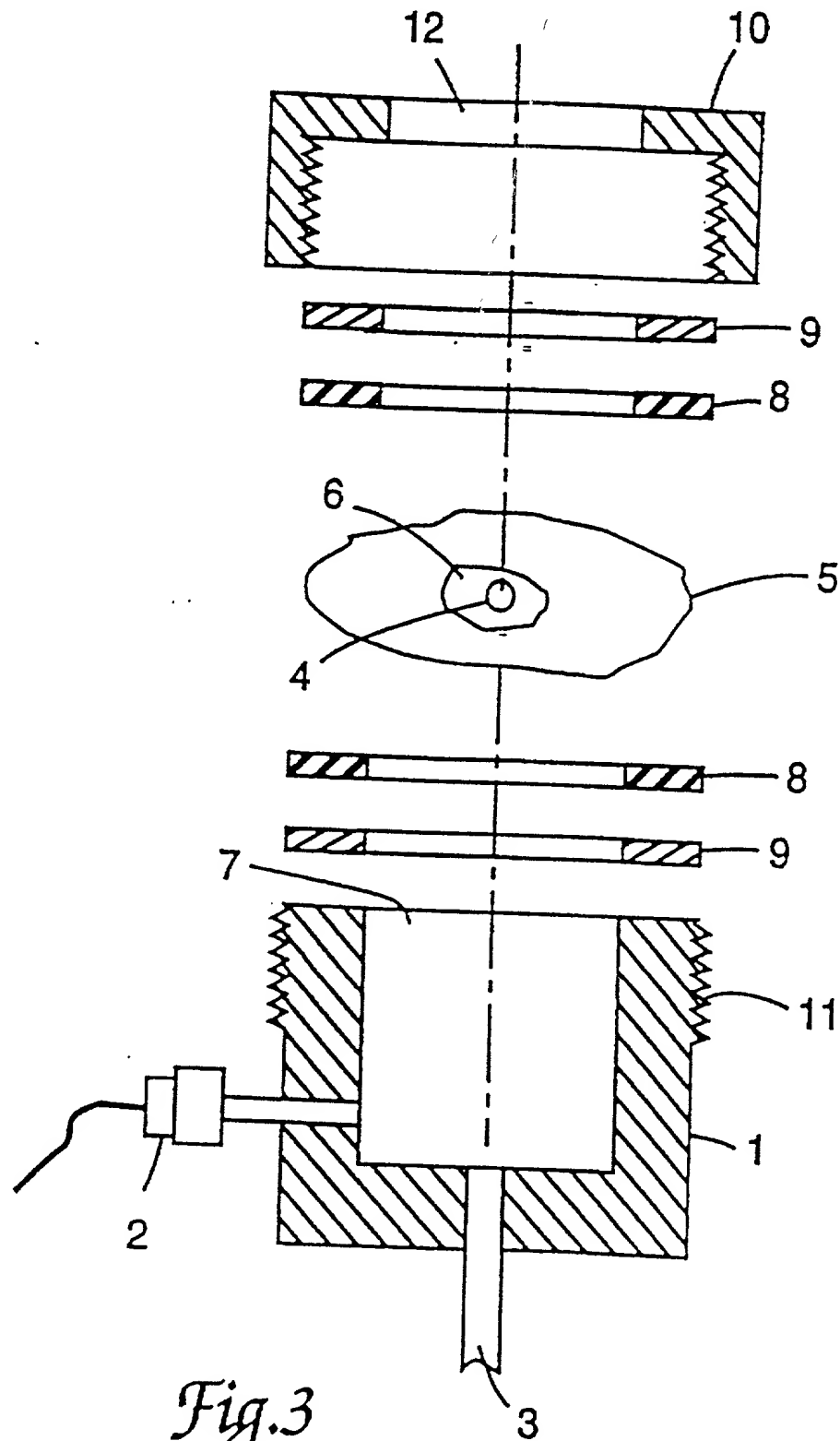


Fig. 1







IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee : Minnesota Mining and Manufacturing Company  
Patent No.: 5,583,114  
Serial No.: 281,473  
Issued : December 10, 1996  
Filed : July 27, 1994  
Title : ADHESIVE SEALANT COMPOSITION

Assistant Commissioner for Patents  
Washington, DC 20231

DECLARATION PURSUANT TO 37 C.F.R. §1.175

We, Thomas H. Barrows, Terry W. Lewis, Myhanh T. Truong, and Matthew T. Scholz, declare that we believe that we are the original and first inventors of the subject matter that is described and claimed in United States Patent No. 5,583,114 for which we solicit a reissue patent; that we have reviewed and understand the contents of the above identified reissue application, including its specification and claims; that we acknowledge the duty to disclose all information of which we are aware that is material to the examination of this reissue application in accordance with Title 37, Code of Federal Regulations (CFR), §1.56(a); that the aforesaid patent is partly inoperative by reason of claiming less than we had the right to claim in the patent; and that said inoperativeness is a result of error that arose inadvertently and without any deceptive intention.

The patent claims less than we had a right to claim, in that the specification of the issued patent discloses an invention that is broader than what is claimed. One error relied upon as the basis of this reissue application was that of originally filing claims directed to subject matter that was narrower than we had a right to claim. This error and any and

"EXPRESS MAIL" Mailing Label Number EL182579374US

Date of Deposit November 4, 1998

I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office To Addressee" with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Maureen T. Whalen  
Maureen T. Whalen

all other errors being corrected in the reissue application arose without deceptive intention.

Matthew T. Scholz is being added as an inventor to the above-referenced patent for which reissue is sought, by a separate petition under 37 C.F.R. §1.324.

Please address communications to:

John J. Gagel, Esq.  
Fish & Richardson, P.C.  
225 Franklin Street  
Boston, MA 02110-2804

Please address all telephone calls to:

John J. Gagel, Esq.  
(617) 542-5070

We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application or any patents issued thereon.

  
Thomas H. Barrows

Residence and Post

Office Address: 62 Shirley Street, Pepperell, Massachusetts,  
01463, USA

Citizenship: United States

Date: 9/5/98

Terry W. Lewis

Terry W. Lewis

3575 Williamsburg Bay  
Woodbury, MN 55129

Residence and Post  
Office Address:

Citizenship: U.S.A.

Date: September 15, 1998

Myhanh T. Truong

Myhanh T. Truong

Residence and Post  
Office Address:

599 Mariner Way  
Woodbury, mn 55129

Citizenship: USA

Date: 9/14/98

Matthew T. Scholz

Matthew T. Scholz

Residence and Post  
Office Address:

8015 Greenbriar Ct.  
Woodbury, MN 55125

Citizenship: USA

Date: 9/14/98

303332.B11

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ADHESIVE SEALANT COMPOSITION, the specification of which

☐ is attached hereto.

☒ was filed on July 27, 1994 as Application Serial No. 07/281,473

and was amended on \_\_\_\_\_.

☐ was described and claimed in PCT International Application No. \_\_\_\_\_  
filed on \_\_\_\_\_ and as amended under PCT Article 19 on \_\_\_\_\_.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: John J. Gagel, Reg No. 33,499, Harold H. Fox, Reg No. 41,498 and Eric L. Prah, Reg No. 32,590

Address all telephone calls to John J. Gagel at telephone number 617/542-5070.

Address all correspondence to John J. Gagel, Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name of Inventor: Thomas H. Barrows

Inventor's Signature: Thomas H. Barrows Date: 9/27/98

Residence Address: Pepperell, Massachusetts

Citizen of: USA

Post Office Address: 62 Shirey Street, Pepperell, Massachusetts 01463

"Express Mail" mailing label number E182579374US

Date of Deposit November 4, 1998

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231

Maureen T. Whalen  
Maureen T. Whalen

**COMBINED DECLARATION AND POWER OF ATTORNEY CONTINUED**

Full Name of Inventor: Terry W. Lewis

Inventor's Signature: Terry W. Lewis

Date: October 2, 1998

Residence Address: Woodbury, Minnesota

Citizen of: USA

Post Office Address: 3575 Williamsburg Bay, Woodbury, Minnesota 55129

Full Name of Inventor: Myhanh T. Truong

Inventor's Signature: Myhanh Truong

Date: 10-2-98

Residence Address: Woodbury, Minnesota

Citizen of: USA

Post Office Address: 599 Mariner Way, Woodbury, Minnesota 55129

Full Name of Inventor: Matthew T. Scholz

Inventor's Signature: Matthew T. Scholz

Date: 10-2-98

Residence Address: Woodbury, Minnesota

Citizen of: USA

Post Office Address: 8015 Greenbriar Court, Woodbury, Minnesota 55125

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee : Minnesota Mining and Manufacturing Company  
Patent No.: 5,583,114  
Serial No.: 281,473  
Issued : December 10, 1996  
Filed : July 27, 1994  
Title : ADHESIVE SEALANT COMPOSITION

Commissioner of Patents and Trademarks  
Washington, DC 20231

STATEMENT OF ASSIGNEE AGREEING TO CORRECTION  
OF INVENTORSHIP UNDER 37 C.F.R. 1.324(b)(3)

By assignment recorded on July 27, 1994 at Reel  
7123/Frames 0460-0461, and an assignment from Matthew T. Scholz,  
a copy of which is attached, the patent is assigned to Minnesota  
Mining and Manufacturing Company.

The undersigned has reviewed all the documents in the  
chain of title of the application and, to the best of  
undersigned's knowledge and belief, title is in the assignee  
identified above.

The undersigned officer of Minnesota Mining and  
Manufacturing Company, whose title is supplied below, is  
empowered to act on behalf of Minnesota Mining and Manufacturing  
Company.

As an officer of the concern to which the above-  
captioned patent has been assigned, the undersigned hereby agrees  
to the change of inventorship of the reissue application of the  
patent from the naming of Thomas A. Barrows, Terry W. Lewis, and

"Express Mail" mailing label number EL18257937405  
Date of Deposit November 4, 1995

I hereby certify that this paper or fee is being deposited with  
the United States Postal Service "Express Mail Post Office to  
Addressee" service under 37 CFR 1.10 on the date indicated above  
and is addressed to the Commissioner of Patents and Trademarks,  
Washington, D.C. 20231

Maureen T. Scholz  
Maureen T. Whalen

Date of Deposit \_\_\_\_\_

I hereby certify under 37 CFR 1.8(a) that this correspondence is being  
deposited with the United States Postal Service as first class mail  
with sufficient postage on the date indicated above and is addressed to  
the Assistant Commissioner for Patents, Washington, D.C. 20231.



Myhanh T. Truong as joint inventors to the naming of Thomas A. Barrows, Terry W. Lewis, Myhanh T. Truong, and Matthew T. Scholz, as joint inventors.

I hereby declare as follows: that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: September 17, 1998

By: 

Name : Gary L. Griswold

Staff Vice President & Chief  
Title: Intellectual Property Counsel

303332.B11